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博 士 学 位 论 文

有毒链状亚历山大藻定量蛋白质组学研究

Quantitative Proteomic Study of a Toxin-producing
Dinoflagellate *Alexandrium catenella*

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摘 要

亚历山大藻是一类在海洋中广泛分布的、能够合成麻痹性贝类毒素(Paralytic shellfish toxins, PSTs)的甲藻。近二十多年来,由亚历山大藻引起的有害(毒)藻华呈全球化拓展的态势,发生频率增加、规模扩大、危害加剧,由亚历山大藻造成的麻痹性贝毒中毒(Paralytic shellfish poisonings, PSPs)事件已经成为一个全球性的环境和健康问题,严重威胁到海洋生态系统的稳定、海洋生物资源的可持续利用和人们的生命财产安全。尽管针对甲藻 PST 开展了大量工作,但由于甲藻庞大的基因组及复杂的基因结构,目前我们对甲藻的产毒机理仍知之甚少。

本论文以有毒链状亚历山大藻(*Alexandrium catenella*)为研究对象,运用基于质谱的定量蛋白质组学技术—iTRAQ(isobaric tags for relative and absolute quantitation)技术,并结合甲藻转录组数据,比较研究了 *A. catenella* 有毒野生株(ACHK-T)与无毒变异株(ACHK-NT)蛋白质组、ACHK-T 不同产毒时期蛋白质组以及细胞代谢抑制剂秋水仙素处理前后 ACHK-T 和 ACHK-NT 蛋白质组,鉴定、筛选差异表达蛋白,确认参与毒素合成的蛋白质及相关的生物学过程,探讨亚历山大藻 PST 合成的分子机制。取得的主要研究结果如下:

(1)运用 iTRAQ 定量蛋白质组学技术结合相应的转录组数据比较研究了正常培养条件下 ACHK-T 和 ACHK-NT 蛋白质组的差异表达。从 *A. catenella* 中鉴定到 3,488 个蛋白质,其中 185 个为差异表达蛋白:106 个在 ACHK-T 中显著高表达,79 个在 ACHK-NT 中显著高表达。ACHK-T 中高表达的蛋白质主要参与碳水化合物代谢、氨基酸及嘌呤合成、蛋白质代谢等过程,包括转录、翻译、翻译后修饰、降解等;而 ACHK-NT 中高表达的蛋白质主要参与叶绿体、过氧化物酶体以及线粒体中的一些过程,包括光合作用、光呼吸、脂肪酸合成及 β 氧化、乙醛酸循环以及活性氧代谢等。这些生物学过程的差异表达表明,ACHK-T 及 ACHK-NT 在碳和能量利用策略上存在差异,这可能与两者不同的产毒能力相关。此外,在 *A. catenella* 的蛋白表达谱中鉴定到了 7 个 PST 合成相关蛋白的 21 个同系物,但这些蛋白质的表达量在 ACHK-T 和 ACHK-NT 之间没有显著差异,产毒相关蛋白可能并非毒素合成过程所特有;

(2)流式细胞及毒素分析结果表明,ACHK-T 在一天内完成一个完整的细

胞周期且 PST 合成主要发生在 G₁ 期末期的两小时内。对 ACHK-T 不同产毒时期细胞蛋白质组的差异表达分析表明, PST 合成可能与蛋白质翻译和叶绿素合成相关。此外, 从 7,232 个鉴定蛋白质中共筛选到包括 *sxtA* 在内的 9 个 PST 产毒相关蛋白的 42 个同系物。但定量结果表明, 在毒素合成最快的时间段内, 除了参与毒素合成过程调控的 *ompR* 蛋白质外, 其它产毒相关蛋白的表达量均没有显著变化。甲藻 PST 合成过程的调控机制可能与蓝藻类似, 参与毒素合成过程的蛋白质可能存在翻译后水平的调节;

(3) 秋水仙素处理显著影响了 *A. catenella* 的细胞结构、活性氧代谢、蛋白质代谢、碳水化合物代谢、生物发光以及叶绿素合成等过程。此外, 秋水仙素处理也显著影响了 *A. catenella* 细胞中多个氮代谢相关蛋白以及细胞周期调控蛋白的表达, 它们的共同作用将 *A. catenella* 抑制在了细胞周期的 G₁ 期。但在氮的吸收利用方面, ACHK-T 和 ACHK-NT 对秋水仙素处理具有不同的响应机制。秋水仙素处理后 ACHK-NT 中参与氮代谢的谷氨酰胺合成酶的表达量显著下调。但在 ACHK-T 中, 其表达量保持相对稳定, 暗示细胞内毒素含量可能对谷氨酰胺合成酶的表达具有反馈调节的作用。为了应对氮限制胁迫, ACHK-T 可能利用蛋白质降解的氮以维持细胞生存, 而将原来用于 PST 合成的氮用于叶绿素的合成。

关键词: 甲藻; 链状亚历山大藻; 麻痹性贝毒; 蛋白质组学; 同位素标记相对和绝对定量; 细胞周期; 秋水仙素

Abstract

Alexandrium is a widely distributed dinoflagellate genus in the ocean and many species within this genus are able to produce paralytic shellfish toxins (PSTs). In the past few decades, with the increase of occurring frequency, expansion scale and serious damage of the PST-producing algal blooms, the paralytic shellfish poisonings (PSPs) caused by PST have become a global environmental health issue which threatens the stability of marine ecosystem, the sustainable development of marine biological resource as well as the human safety. Much effort has been devoted to the toxin biosynthesis but the biosynthetic mechanism in dinoflagellates remains obscure owing to our poor understanding of the massive genomes and unique chromosomal characteristics.

This study, for the first time, applied the mass spectrometry-based quantitative proteomic approach: isobaric tags for relative and absolute quantitation (iTRAQ) combined with the transcriptomic database, to compare the proteomes of a toxin-producing dinoflagellate *Alexandrium catenella* (ACHK-T) and its non-toxic mutant (ACHK-NT), the proteomes of different toxin-producing stages of ACHK-T and the proteomes of ACHK-T and ACHK-NT with and without colchicine treatment, respectively, identified and screened differentially displayed proteins, characterized toxin-related proteins and biological processes, and discussed PST biosynthetic mechanism in dinoflagellates. The main results were as follows:

(1) The differentially expressed proteomes of ACHK-T and ACHK-NT were compared using a combination of iTRAQ-based proteomic approach and the transcriptomic data. 3,488 proteins were identified from *A. catenella*, 185 of them were differentially displayed: 106 proteins mainly involved in amino acid and purine biosynthesis, protein and carbohydrate metabolism and bioluminescence were more abundant in ACHK-T while 79 proteins mainly participated in photosynthesis, fatty acid biosynthesis and the peroxisome processes displayed higher abundances in ACHK-NT. The variations of these biological processes suggested that the carbon and energy utilization strategies were different between these two strains, which might be related to the different toxin biosynthesis abilities. In addition, 21 homologs of seven toxin-related proteins were identified but they varied insignificantly between the two strains, suggesting that they were not unique to the toxin biosynthesis process.

(2) Flow cytometry and toxin analysis showed that ACHK-T completed a cell cycle within one day, and PST synthesis occurred mainly within 2 hours of the late G₁ phase. Quantitative results indicated that PST biosynthesis might be related to protein translation and chlorophyll biosynthesis. In addition, a total of 42 homologs of nine toxin-related proteins were obtained from 7,232 proteins identified, but none of them, except for the regulative protein ompR, displayed differentially during the fastest toxin biosynthesis period. It is, therefore, regulation mechanism of PST biosynthesis in dinoflagellates might be similar to cyanobacteria and the toxin-related proteins in dinoflagellates might suffer post-translational regulation.

(3) Colchicine treatment significantly influenced the cell structure, bioluminescence, chlorophyll biosynthesis, reactive oxygen metabolism, proteins and carbohydrate metabolism of *A. catenella*. In addition, colchicine also significantly affected the expressions of several nitrogen-metabolic proteins and cell-cycle regulative proteins related to cellular G₁/S phases transition. Their interaction might arrest the cell cycle in G₁ phase. However, for the absorption and utilization of nitrogen, ACHK-T and ACHK-NT responded differently to the colchicine-treatment. Colchicine significantly inhibited the expression of glutamine synthetase (GS) in ACHK-NT while GS expression in ACHK-T maintained relatively stable, suggesting intracellular toxin content might play a feedback role in regulating GS expression. As a response to nitrogen-deprivation stress, ACHK-T might reuse the nitrogen degraded from the proteins to maintain the cell survival, and utilize nitrogen originally for PST biosynthesis to chlorophyll biosynthesis.

Keywords: Dinoflagellate; *Alexandrium catenella*; Paralytic shellfish toxins; Proteomics; iTRAQ; Cell cycle; Colchicine

缩略词表

缩写	英文名称	中文名称
2D-DIGE	Two-dimensional difference gel electrophoresis	荧光差异凝胶双向电泳
2-DE	Two dimensional electrophoresis	双向电泳
ACN	Acetonitrile	乙腈
ACP	Acyl carrier protein	酰基载体蛋白
ATP	Adenosine triphosphate	三磷酸腺苷
BLAST	Basic local alignment search tool	基于局部比对算法的搜索工具
BSA	Bovine serum albumins	牛血清蛋白
CCMA	Center for collections of marine algae	海洋藻类收集中心（厦门）
CDS	Coding sequence	编码序列
CHAPS	3-[(3-Cholamidopropyl) dimethylammonio] propanesulfonic acid	3-[(3-胆酰胺基丙基)二甲基铵基]-1-丙磺酸
CoA	Coenzyme A	辅酶 A
COG	Clusters of Orthologous Groups of proteins	蛋白质直系同源数据库
DNA	Deoxyribonucleic acid	脱氧核糖核酸
DTT	Dithiothreitol	二硫苏糖醇
FA	Formic acid	甲酸
GO	Gene Ontology	基因本体注释
GTX	Gonyautoxin	膝沟藻毒素
HGT	Horizontal gene transfer	基因横向转移
HPLC	High performance liquid chromatography	高效液相色谱
IAM	Iodoacetamide	碘乙酰胺
iTRAQ	isobaric Tags for Relative and Absolute Quantitation	同位素标记相对和绝对定量
KEGG	Kyoto Encyclopedia of Genes and Genomes	京都基因与基因组百科全书
LBP	Luciferin binding protein	荧光素结合蛋白
LCF	Luciferase	荧光素酶
NCBI	National Center for Biotechnology Information	国家生物技术信息中心（美国）
PBS	Phosphate buffered saline	磷酸盐缓冲液
PSP	Paralytic shellfish poisoning	麻痹性贝毒中毒
PST	Paralytic shellfish toxin	麻痹性贝类毒素
SAM	S-adenosylmethionine	活性腺苷甲硫胺酸
SCX	Strong cation exchange	强阳离子交换
STX	Saxitoxin	石房蛤毒素
TCA	Tricarboxylic acid	三羧酸
TEAB	Tetraethyl-ammonium bromide	溴化四乙铵
Tris	Hydroxymethyl aminomethane	三羟甲基氨基甲烷

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